X-ray crystallography of the complex of SeqA, a negative regulator of replication, and a hemi-methylated DNA

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Escherichia coli chromosomal DNA has approximately 1900 GATC sequences, which are methylated by Dam methyltransferase. In the DNA replication step, until the synthesizing dauter strand is methylated by Dam methyltransferase, the chromosomal DNA is kept in the hemimethylated state. *Escherichia coli* SeqA protein binds to the hemimethylated sequence in the *ori* region to restrict one cycle of replication per one cycle of cell division. To elucidate the mechanism how SeqA strictly recognizes the hemimethylated DNA, we crystallized the complex of SeqA and 10 bp or 16 bp DNA including hemimethylated adenosine. Crystals of the complex with 16 bp hemimethylated DNA belong to the monoclinic spacegroup *C*2 (a=84.19 Å, b =67.98 Å, c = 88.21 Å, β =110.6°), and diffract X-ray up to 2.65 Å resolution. The Rmerge and the completeness were 0.038 (0.114) and 91.1% (57.1%), respectively (the values at the outer shell are shown in parentheless). However, the Wilson plot implies that the crystals are twined, and we could not determined the phase by Se-MAD phasing. On the other hand, crystals of the complex with 10 bp DNA belong to the hexagonal spacegroup *P*622 (a=b =152.96 Å, c = 119.1 Å), and diffracts X-ray beyond 3.0 Å resolution. By MAD method using SeMet derivative, we determined the phase and builtg the atomic model. The structure reveals that symmetrical Thr-Asn-Thr-Ans-Thr sequence plays a crucial role in the recognition of hemi-methylated double-stranded DNA.

X-ray crystallography of the complex of tRNA and CCA-adding enzyme that repairs the 3'CCA terminus of tRNA

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The 3'-terminal CCA sequence (positions 74, 75, and 76) found in all mature tRNAs is essential for various aspects of gene expression in all organisms. The CCA sequence is required for the aminoacylation by aminoacyl-tRNA synthetase, and for the peptide-bond formation on ribosomes. The CCA sequence at the 3' end of tRNAs is repaired and sometimes constructed *de novo* by a CCA-adding enzyme [ATP (CTP): tRNA nucleotidyltransferase] using CTP and ATP as substrates. The CCA-adding enzyme is present in all three primary kingdoms — eubacteria, eukarya, and archaea, indicating the conservation of its activity throughout evolution. The CCA-adding enzyme is indispensable in many organisms in which some or all of the tRNA genes do not encode CCA. Otherwise, the activity is advantageous for cell viability in the other organisms, where all tRNA genes encode CCA.
The CCA-adding enzyme is a member of the nucleotidyltransferases (NTs) family, which encompasses enzymes as diverse as poly(A) polymerase (PAP), terminal deoxynucleotidyltransferase (TdT), DNA polymerase β (pol β), glutamine synthase adenylyltransferase, and kanamycin nucleotidyltransferase (KNT). The CCA-adding enzyme is a remarkable enzyme among the NT family; it synthesizes the ordered CCA sequence at the 3' end of the tRNA primer without the aid of a nucleic acid template. Furthermore, the enzyme is sensitive to register; it strictly monitors the status of the tRNA 3' end to reconstruct the complete CCA terminus. Based on biochemical and biophysical studies, several models have been proposed to explain the specific activity of this remarkable RNA polymerase. However, the details of the mechanism for the CCA addition have been elusive for three decades.

To elucidate how CCA-adding enzyme attaches the defined CCA trinucleotide onto all tRNA species, we crystallized *Aquifex aeolicus* CCA-adding enzyme complexed with tRNA transcript. The crystals diffract X-ray beyond 2.8 Å resolution, and belong to a trigonal space group $P2_1$ with unit-cell parameters a =60.3Å, b = 123.0 Å, c = 110.1 Å, and β=91.2°. The Rmerge and completeness were 0.082 (0.293) and 95.4% (89.2%), respectively (the values at the outer shell are shown in parentheless). We also collected MAD data of crystals of the seleno-methionylated protein complex. The solved structure elucidated how the enzyme adds the defined RNA sequence onto the 3'-terminus of a primer tRNA without any aid of DNA template.